

Variation in lipid and fatty acid composition of two *Aspergillus* species grown on glucose or dodecane

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ABSTRACT

Two species of *Aspergillus*, *A. terreus* and *A. sulphureus*, were grown in an inorganic medium provided with either glucose or dodecane as sole source of carbon. Their total lipids and sterols were extracted and analyzed. Dodecane-grown fungi contained more total lipids and sterols than glucose-grown fungi. Dodecane-grown mycelia accumulated substantial amounts of triacylglycerols, fatty acids, sterols and diacylglycerophosphoethanolamines. The major acyl moieties in total lipids from glucose-grown fungi were palmitic, oleic, and linoleic acids. In addition, small amounts of palmitoleic and stearic acids were also present and were confined to diacylglycerophosphoethanolamines. Dodecane-grown mycelia contained higher concentrations of fatty acids with chain length less than 16 carbon atoms in their total lipids than did glucose-grown fungi. These fatty acids were mainly present in the free form. In all extracts traces of linolenic acid and fatty acids with longer chains were also detected. The major sterol was ergosterol; lesser amounts of lanosterol and calciferol were detected. Dodecane-grown fungi contained more ergosterol than glucose-grown fungi.

INTRODUCTION

Many reports have described the effect of hydrocarbons on the lipid and fatty acid composition of hydrocarbon-utilizing bacteria and yeasts (Makula & Finnerty 1968a, 1968b, 1970, 1972; Thorpe & Ratledge 1972; Hug *et al.* 1974). Some work has also been published on the effect of hydrocarbons on the fatty acid composition of hydrocarbon-utilizing fungi (Cooney & Proby 1971; Cerniglia & Perry 1974; Kan & Cooney 1975). Cerniglia & Perry (1974) showed that *Cunninghamella elegans* and *Penicillium zonatum* contain considerable amounts of fatty acids with the same carbon chain as the hydrocarbon substrate. This is expected since the initial step of alkane assimilation involves its oxidation to the corresponding fatty acids (Cerniglia & Perry 1974; Siporin & Cooney 1975; Ascenzi & Vestal 1979). On the other hand, *Cladosporium resinae* cultured on a series of n-alkanes does not seem to incorporate the substrate hydrocarbons directly after oxidation to the corresponding fatty acids (Cooney & Proby 1971; Kan & Cooney 1975). It is believed that this organism

metabolizes the products of initial oxidation via β -oxidation to acetyl CoA and synthesizes cellular fatty acids *de novo* (Cooney & Proby 1971).

Some studies deal with the lipid class composition of alkane-utilizing filamentous fungi. According to Kan & Cooney (1975) cellular lipids of *Cladosporium resinae* contain diacylglycerophosphoethanolamines (PE) and diacylglycerophosphocholines (PC) as their major phospholipids, with lesser amounts of diacylglycerophosphoserines and traces of a cardiolipin-like compound. Miyazima *et al.* (1985a, 1985b) showed that the lipid classes that increase most by growth of *Aspergillus* sp. on n-alkane series are triacylglycerols and cardiolipins.

The present paper describes the lipid and fatty acid composition of two species of *Aspergillus* grown on glucose and n-dodecane as sole sources of carbon and energy.

MATERIALS AND METHODS

1. MICROORGANISMS AND CULTURAL CONDITIONS

Aspergillus terreus and *A. sulphureus* were isolated from a soil sample contaminated with crude oil obtained from an oil field in Kuwait. The fungi were identified by consulting Raper & Fennell (1977) and Domsch *et al.* (1980). Stock cultures were maintained on slopes of chemically defined medium (CDM) (Sorkhoh *et al.* 1990) supplemented with 1% v/v filter-sterilized crude oil as well as on potato dextrose agar (PDA) slants, and were subcultured onto fresh medium every 4 to 5 weeks. The two *Aspergillus* spp. were grown on a rotary shaker (160 rpm) at 25°C in 50 ml samples of potato dextrose broth for 7 days. Cells were collected by centrifugation, washed 3 times with 0.9% NaCl (w/v) and suspended in 20 ml saline. This was used to inoculate 2.5 l of CDM supplemented with 1% (w/v) either glucose or dodecane. The CDM was supplemented with 10 ml/l vitamin solution composed of (mg/l): 0.01 biotin, 2.0 pyridoxin, 2.0 nicotinic acid, 2.0 thiamine and 10.0 meso-inositol; pH 5.6. The cultures were incubated on a shaker at 25°C for 14 days, and the biomass determined following filtration and drying.

2. LIPID EXTRACTION AND ANALYSIS

The fungal biomass was resuspended in propan-2-ol and incubated at 70°C for 45 min; this step was included in order to inactivate degradative enzymes such as phospholipases (Hitchcock *et al.* 1986) and extract part of the lipids. The suspension was cooled to 25°C, re-centrifuged and the supernatant added to the total lipid extract. The pellet was then extracted with chloroform/methanol (2:1, v/v) and purified using established procedures (Folch *et al.* 1957). The combined extracts were analyzed by thin layer chromatography (TLC) on plates of silica gel G. Apolar compounds were resolved by the solvent system hexane/diethyl ether/acetic acid (75:25:1 by vol.) (Mangold & Malins 1960). Polar lipids were analyzed by uni-dimensional chromatography using chloroform/methanol/water (65:25:4, by vol.). The lipid fractions were visualized with iodine vapour or by charring at 220°C after spraying the plates with 50% (v/v) H₂SO₄. Individual classes were identified by comparing their chromatographic behaviour with that of authentic samples and by

Table 1. Lipid and sterol contents* of *Aspergillus* spp. grown on glucose or dodecane as sole carbon source.

	<i>A. terreus</i>		<i>A. sulphureus</i>	
	glucose	dodecane	glucose	dodecane
Lipids	0.48 ± 0.03	13.60 ± 0.70	1.20 ± 0.08	4.70 ± 0.10
Sterols	0.12 ± 0.02	0.35 ± 0.04	0.10 ± 0.01	0.29 ± 0.01

* As % of the dry weight. Each value is the mean ± SD of three separate determinations.

using specific spray reagents (Dittmer & Lester 1964; Siakotos & Rouser 1965; Krebs *et al.* 1967).

The IR spectra of fractions isolated by preparative TLC were recorded using a Perkin-Elmer 398 IR spectrophotometer and compared with the spectra of authentic samples. Lipid fractions resolved by TLC and subjected to charring were quantified densitometrically using a Beckman R-112 densitometer.

Samples of apolar and polar lipid fractions separated by preparative TLC were subjected to methanolysis (Chalvardjian 1964). The resulting methyl esters were purified by TLC and analyzed by gas liquid chromatography (GLC) using a Pye-Unicam model 204 gas chromatograph fitted with a glass column (1.83 m × 4 mm i.d.) packed with 15% diethyleneglycolsuccinate (DEGS) on Anakrom D, 100–120 mesh, provided with a flame ionization detector, temperature of injection 200°C, column and detector 180°C.

3. STEROLS EXTRACTION AND ANALYSIS

Sterols were extracted according to the method of Ghannoum *et al.* (1990). KOH (1.5 g) in 2 ml of distilled water was added to 0.2 g wet biomass and the volume of the suspension adjusted to 10 ml by the addition of ethanol. The solution was refluxed under nitrogen for 3 h, diluted with an equal amount of water and extracted with four volumes of heptane. The extract was washed with water, dried over anhydrous Na₂SO₄ and evaporated *in vacuo* to give the total sterols.

The sterols were fractionated by TLC using silica gel G plates (0.25 mm) and 40–60 petroleum ether : diethyl ether (3 : 1, v/v) as a developing solvent. The Lifschutz reagent [concentrated sulphuric acid : glacial acetic acid (1 : 1, v/v)] was used to detect sterols as red or purple spots. Fractions separated by TLC were identified by comparison of their R_f values with commercially available standards. Preparative TLC was used to separate the individual sterols ready for identification using various spectroscopic methods.

Silylated sterols were prepared according to Vandenhuevel & Court (1968). Up to 1 mg samples of sterol extract were reacted in a glass-stoppered flask with 50 μl of 10% trimethyl chlorosilane in chloroform (v/v), the reagents being added in that order. Brief mixing by stirring was applied after each addition. The reaction mixture was left at room temperature for at least 4 h. Excess solvent and reagents were removed using the method of Vandenhuevel *et al.* (1965). Fifty μl of CS₂ were then added to the flask to dissolve the reaction mixture. Portions (0.5 μl) were loaded on

Table 2. Lipid composition of *Aspergillus terreus* and *A. sulphureus* grown on glucose or dodecane as sole source of carbon and energy

Lipid class	<i>A. terreus</i>		<i>A. sulphureus</i>	
	glucose	dodecane	glucose	dodecane
<i>Apolar lipids</i>				
Steryl esters	6.2 ± 0.8	Tr	Tr	Tr
Triacylglycerols	20.7 ± 1.1	27.8 ± 0.3	8.6 ± 0.2	15.2 ± 0.9
Fatty acids	10.3 ± 0.4	15.5 ± 0.4	13.5 ± 0.8	17.4 ± 1.0
1,2 and 1,3-diacylglycerols	7.6 ± 0.2	10.0 ± 0.5	11.5 ± 0.4	9.5 ± 0.6
Sterols	9.7 ± 0.6	14.7 ± 0.8	7.9 ± 0.2	9.5 ± 0.4
1- and 2-monoacylglycerols	14.5 ± 1.2	8.4 ± 0.7	16.7 ± 0.9	8.2 ± 0.7
<i>Polar lipids</i>				
Diacylglycerophosphoethanolamines	7.6 ± 0.6	9.1 ± 0.4	8.2 ± 0.3	9.8 ± 0.2
Diacylglycerophosphoglycerols	6.8 ± 0.2	3.2 ± 0.6	7.4 ± 0.3	6.3 ± 0.9
Diacylglycerophosphocholines	16.6 ± 0.1	11.3 ± 0.4	26.2 ± 1.2	24.1 ± 0.2

Values are expressed as % (w/w) of total lipids; each value is the mean ± SD of three determinations.
Tr = Trace.

SP2100 column (3% on 100/120 gaschrome Q) in a Pye-Unicam gas chromatograph (series 204). The retention times of the peaks were compared with those of reference standards. The samples were eluted at 230°C and lipids detected with flame ionization detector. Individual components were identified by comparison of the retention times relative to ergosterol (Rerg) with those of commercially available standards.

Table 3. Constituent fatty acids* of total lipids from *Aspergillus terreus* and *A. sulphureus* grown on glucose or dodecane as sole carbon source

Fatty acids	<i>A. terreus</i>		<i>A. sulphureus</i>	
	glucose	dodecane	glucose	dodecane
< 16:0	Tr	2.3 ± 0.1	Tr	2.1 ± 0.3
16:0	15.3 ± 0.8	14.1 ± 0.3	16.4 ± 0.4	14.0 ± 1.0
16:1	6.3 ± 1.1	Tr	Tr	Tr
18:0	4.2 ± 0.2	14.0 ± 0.5	4.0 ± 0.6	11.2 ± 0.8
18:1	23.8 ± 1.8	25.1 ± 0.9	8.0 ± 0.7	28.2 ± 1.5
18:2	50.4 ± 2.1	44.5 ± 2.3	71.6 ± 2.1	44.5 ± 2.6
18:3	Tr	Tr	Tr	Tr
> 18:3	Tr	Tr	Tr	Tr
Ratio	4.1	2.5	3.9	2.9
unsaturated: saturated, without < 16:0 and > 18:3				

* Values are expressed as % (w/w) of the constituent fatty acids; each value is the mean ± SD of three determinations.

Tr = Trace.

RESULTS

1. LIPID COMPOSITION

The total lipid and sterol contents of dodecane-grown fungi were higher than those of glucose-grown fungi (Table 1). The relative yield of lipids and sterols for dodecane-grown *A. terreus* was 28.3 and 2.9 fold higher and of *A. sulphureus* 3.8 and 1.6 fold higher, respectively, than in fungi grown on glucose.

The quantitative measurements showed that all extracts contained more apolar than polar lipids (Table 2). Apolar lipid classes in the various extracts were steryl esters, sterols, free fatty acids and tri-, di- and mono-acyl glycerols. In both species dodecane-grown cells accumulated substantially greater amounts of triacylglycerols, fatty acids and sterols, while the amounts of monoacylglycerols decreased. Polar lipid classes in all extracts were diacylglycerophosphoethanolamines (PE), diacylglycerophosphocholines (PC) and diacylglycerophosphoglycerols (PG). Dodecane-grown cells of both species contained higher percentage of PE than glucose-grown cells. Compared with glucose-supplemented medium, dodecane reduced the amounts of PC and PG in the lipid fraction of *A. terreus* but did not significantly affect these classes in *A. sulphureus*.

2. FATTY ACID PATTERNS

The fatty acid patterns of total lipids from glucose and dodecane-grown mycelia are shown in Table 3. The predominant acyl moieties in all extracts were palmitic (16 : 0), oleic (18 : 1) and linoleic (18 : 2) acids. Lesser amounts of stearic acid (18 : 0) were detected in glucose-grown cells. Traces of linolenic acid and fatty acids higher than 18 : 3 were detected in all extracts. Lipids from dodecane-grown mycelia of both species contained relatively more of the fatty acids with chain length shorter than 16 carbon atoms than lipids from glucose-grown cells, higher proportions of stearic acid and decreased proportions of linoleic acid. Oleic acid showed little change in *A. terreus* but increased three-fold in *A. sulphureus* on dodecane, compared to glucose-containing medium. Correspondingly, in both species, lipids of dodecane-grown mycelium were less unsaturated than those of mycelium growing on glucose.

The data in Table 4 show the fatty acid patterns of individual lipid classes. The phospholipid classes, particularly diacylglycerophosphocholines and free fatty acids of glucose-grown mycelia of *A. terreus* and *A. sulphureus* contained appreciable proportions of linoleic acid (18 : 2) which decreased (as percent of constituent fatty acids) when the fungi were grown on dodecane. In almost all lipid classes, the percent of stearic (18 : 0) and oleic (18 : 1) acids increased on dodecane, while in PG the percent of oleic acid (18 : 1) decreased. In both species, palmitoleic acid (16 : 1) occurred in considerable amounts only in PE and decreased as a percentage of PE fatty acids when the fungi were grown on dodecane. The highest concentrations of fatty acids shorter than C₁₆ were detected in the free fatty acid fractions isolated from dodecane-grown mycelia.

3. STEROLS

The examination of the sterol fraction of *A. terreus* and *A. sulphureus* showed that the major sterol present was ergosterol which increased in dodecane-grown fungi.

Table 4. Constituent fatty acids of individual lipid classes from *Aspergillus terreus* and *A. sulphureus* grown on glucose or dodecane as sole source of carbon

Fatty acids	<i>A. terreus</i>		<i>A. sulphureus</i>	
	glucose	dodecane	glucose	dodecane
Diacylglycerophosphocholines:				
< 16:0	Tr	Tr	Tr	Tr
16:0	9.4 ± 0.8	28.2 ± 1.2	18.7 ± 0.5	15.5 ± 0.8
16:1	Tr	Tr	Tr	Tr
18:0	8.4 ± 0.3	5.7 ± 0.2	Tr	4.6 ± 0.4
18:1	19.0 ± 1.0	20.0 ± 0.9	15.3 ± 0.7	25.3 ± 1.2
18:2	63.2 ± 1.8	46.1 ± 1.7	66.0 ± 0.9	54.6 ± 1.4
18:3	Tr	Tr	Tr	Tr
> 18:3	Tr	Tr	Tr	Tr
Diacylglycerophosphoethanolamines:				
< 16:0	Tr	Tr	Tr	Tr
16:0	16.9 ± 0.9	18.1 ± 0.3	22.6 ± 1.4	18.0 ± 0.7
16:1	20.3 ± 0.6	9.2 ± 0.9	25.8 ± 1.5	3.7 ± 0.1
18:0	7.4 ± 0.5	9.4 ± 0.2	5.6 ± 0.3	8.8 ± 0.8
18:1	18.0 ± 0.7	25.6 ± 1.0	14.0 ± 1.0	30.2 ± 2.0
18:2	37.4 ± 0.8	37.7 ± 1.3	32.0 ± 2.1	39.3 ± 1.8
18:3	Tr	Tr	Tr	Tr
> 18:3	Tr	Tr	Tr	Tr
Diacylglycerophosphoglycerols:				
< 16:0	Tr	Tr	Tr	Tr
16:0	22.4 ± 0.4	15.3 ± 1.0	10.2 ± 0.4	20.3 ± 0.8
16:1	Tr	Tr	Tr	Tr
18:0	Tr	5.6 ± 0.2	Tr	Tr
18:1	22.7 ± 0.6	18.8 ± 0.7	33.6 ± 1.6	27.5 ± 0.9
18:2	42.4 ± 1.8	42.5 ± 1.2	56.2 ± 2.4	52.2 ± 1.3
18:3	Tr	Tr	Tr	Tr
> 18:3	12.5 ± 0.3	17.8 ± 0.8	Tr	Tr
Triacylglycerols:				
< 16:0	Tr	Tr	Tr	Tr
16:0	16.0 ± 0.8	23.1 ± 0.3	14.9 ± 0.5	11.1 ± 0.1
16:1	Tr	Tr	2.4 ± 0.1	1.4 ± 0.3
18:0	15.7 ± 0.8	25.7 ± 1.3	Tr	1.2 ± 0.1
18:1	23.8 ± 0.5	28.0 ± 0.9	60.8 ± 0.5	65.8 ± 1.3
18:2	25.2 ± 1.0	23.0 ± 0.4	21.9 ± 0.3	20.0 ± 0.6
18:3	Tr	Tr	Tr	Tr
> 18:3	19.3 ± 0.6	0.2 ± 0.01	Tr	0.5 ± 0.03
Free fatty acids:				
< 16:0	Tr	12.4 ± 1.2	Tr	9.4 ± 0.6
16:0	15.8 ± 0.1	16.5 ± 0.2	17.8 ± 0.8	13.0 ± 0.6
16:1	Tr	Tr	Tr	Tr
18:0	6.8 ± 0.8	16.0 ± 1.3	2.0 ± 0.3	12.4 ± 0.7
18:1	16.4 ± 0.4	21.8 ± 0.7	15.9 ± 1.2	26.7 ± 0.9
18:2	61.0 ± 2.4	33.3 ± 1.6	64.3 ± 2.8	35.4 ± 2.0
18:3	Tr	Tr	Tr	Tr
> 18:3	Tr	Tr	Tr	3.1 ± 0.4

Values are expressed as % (w/w) of the constituent fatty acids; each value is the mean ± SD of three determinations.

Tr = Trace.

Table 5. Sterol composition of *Aspergillus terreus* and *A. sulphureus* grown on glucose or dodecane as carbon source*

Sterol	Rerg*	<i>A. terreus</i>		<i>A. sulphureus</i>	
		glucose	dodecane	glucose	dodecane
Squalene	0.35	2.5 ± 0.3	Tr	Tr	Tr
Calciferol	0.83	1.4 ± 0.1	0.3 ± 0.03	1.4 ± 0.2	0.4 ± 0.05
Ergosterol	1.00	81.9 ± 1.1	93.6 ± 1.9	88.2 ± 1.6	93.7 ± 1.9
Lanosterol	1.29	14.2 ± 1.3	6.1 ± 0.5	10.4 ± 0.8	5.9 ± 0.3

* Retention time relative to ergosterol = 1.000. Relative retention time for different 'runs' varied less than ± 0.006.

Tr = Trace.

Data are expressed in % of total sterols.

Smaller amounts of 4,4-dimethyl sterols (lanosterol) were detected. There was a decrease in the percentage of the latter sterol as well as in squalene in the two fungi grown on dodecane (Table 5).

DISCUSSION

The results of the present study show that the lipids of *A. terreus* and *A. sulphureus* grown on glucose consist of a mixture of glycerophospholipids, steryl esters, sterols, free fatty acids and tri-, di- and monoacylglycerols. The same lipid classes are present when the two species are grown on dodecane as sole source of carbon and energy, except that these cells accumulate more fatty acids, triacylglycerols, sterols and PE. Miyazima *et al.* (1985a, 1985b) working with *Aspergillus* sp. showed a similar increase in free fatty acids, triacylglycerol and PE during growth on dodecane. It is believed that the increase in the free fatty acid levels in dodecane-grown cells is due to the direct oxidation of the alkane substrate to its corresponding fatty acid (Cerniglia & Perry 1974; Siporin & Cooney 1975; Ascenzi & Vestal 1979). The present results support this view. Higher levels of fatty acids of chain length shorter than C₁₆ were found in the free fatty acid fraction of dodecane-grown cells.

The increase in sterol levels in dodecane-grown cells is quite interesting because of the significance of this lipid class in membrane stability. Membrane lipids are known to be involved in several functions including active transport (Prasad & Rose 1986). From the results indicating an increase in the ergosterol levels in dodecane-grown cells, it may be suggested that sterols in the *Aspergillus* spp. studied here form hydrophobic sites in the cell membrane through which the alkane is transported to the cytoplasm. Ergosterol is the major sterol present in both *Aspergillus* spp. The increase in ergosterol was accompanied by a relative decrease in the level of lanosterol and squalene, suggesting that ergosterol biosynthesis from squalene proceeds at a higher rate on hydrocarbons than on glucose (Van den Bossche *et al.* 1978).

The phospholipids : sterol ratio is believed to exert an important role in membrane function (Gottlieb & Show 1967). Our results show that this ratio in both *Aspergillus* spp. is lower on n-dodecane than on glucose-grown mycelia. This change, together with the observed decrease in the ratio of unsaturated to saturated

fatty acids, should increase membrane stability and may be necessary for growth in the presence of dodecane. It is, however, difficult to predict a unified mechanism for hydrocarbon up-take since the growth in the presence of hydrocarbons results in a complex dynamic situation, rather than a simple one.

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اختلاف في تركيب الليبيدات والأحماض الدهنية في نوعين
من جنس أسبرجلس ناميين على الجلوكوز
أو الدوديكان

ناصر صرخوه

قسم النبات والميكروبيولوجيا بجامعة الكويت ،
ص . ب . ٥٩٦٩ ، الصفاة ١٣٠٦٠ ، الكويت

خلاصة

زرع نوعان من أسبرجلس وهما أسبرجلس تيريس وأسبرجلس سلفيوريس في وسط غذائي غير عضوي به إما جلوكوز أو دوديكان كمصدر وحيد للكربون . ثم استخلصت الليبيدات الكلية من هذه الفطريات وحللت . وقد وجد ان الفطريات النامية في وجود دوديكان تحتوي على كميات من الليبيدات الكلية واستيرولات أكبر من تلك الموجودة في الفطريات النامية في وجود جلوكوز . ووجد أن الفطريات النامية على دوديكان تحتوي على كميات محسوسة من جلسيريدات ثلاثية وأحماض دهنية وستيرولات وفسفاتيديل وإيثانولامينات ، وكانت أعلى الأحماض الدهنية تركيزا في الليبيدات الكلية من الفطريات النامية على الجلوكوز هي أحماض پلميتيك وأوليك ولينوليك . إضافة لذلك كانت هناك كميات قليلة من حمضي پلميتوليك وستياريك ، واقتصر وجودهما على فوسفاتيديل إيثانولامينات . واحتوت الفطريات النامية على دوديكان على نسب أدنى من الأحماض الدهنية ذات سلاسل ثقل في الطول عن ١٦ ذرة كربون في ليبيداتها الكلية ، وذلك مقارنة بالفطريات النامية على جلوكوز . وكانت هذه الأحماض الدهنية موجودة في صورة طليقة . ووجدت في كل المستخلصات بقايا من حمض لينولينك وأحماض دهنية أخرى ذات سلاسل كربونية أطول من ذلك . وكان الستيرول الرئيسي هو إرجوستيرول . وكانت هناك كميات أقل من لانوستيرول وكلسيفيرول ، واحتوت الفطريات النامية على دوديكان على كميات من إرجوستيرول أكبر من تلك التي تحتويها الفطريات النامية على جلوكوز .